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A review on harvesting, oil extraction and biofuels production technologies from microalgae

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ABSTRACT

Microalgae are receiving increasing attention worldwide as an alternative and renewable source for energy production. Through various conversion processes, microalgae can be used to produce many different kinds of biofuels, which include biodiesel, bio-syngas, bio-oil, bio-ethanol, and bio-hydrogen. However, large scale production of microalgal biofuels, via many available conversion techniques, faces a number of technical challenges which have made the current growth and development of the algal biofuel industry economically unviable. Therefore, in addition to algae culture and growth, it is also essential to develop cost-effective technologies for efficient biomass harvesting, lipid extraction and biofuels production. This review aims to collate and present an overview of current harvesting, oil extraction and biofuels production technologies from microalgae. Since much of the current studies on oil extraction are focused on biodiesel production from microalga, this study, apart from discussing the various biodiesel production techniques in the later sections, has also done a detailed discussion on the production techniques of other biofuels.

Contents

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1. Introduction

Critical issues like unsustainable and diminishing fossil fuel resources, and their adverse environmental impacts due green house gases (GHG) addition into the climate, have called for development of techniques and policies to enhance uses and production of renewable energy sources [1]. Increasing concerns about sustainability and the environment have led to a common practice to reduce carbon dioxide emissions and thus global warming, resulting from human activities [2]. Biofuels, produced from biomass [3], are one of the most feasible, renewable and alternate energy resources to deal with the above issues. The benefits include sustainability, reduction of GHG emissions [4], reduced environmental impact [3] and greater energy security [4,3]. There are many different types of biofuels, produced from biomass, such as vegetable oils, biodiesel, bio-ethanol, bio-syngas, bio-oil, and bio-hydrogen [3].

India consumes five times more diesel than gasoline [5], and biodiesel being the best candidate for diesel fuels [6], has thus attracted much attention as a blending component or a direct replacement for diesel fuel for transportation [6,7]. Biodiesel, being renewable and environmentally friendly fuel, has recently been considered as one of the best alternative resources of fossil fuels [8].

From an environmental perspective, benefits of biodiesel include, reduction of carbon monoxide, carbon dioxide [9], and sulfur emissions into the atmosphere [2,6]. Moreover, it is non-toxic and biodegradable [8,9,2,6]. It has similar energy content, chemical and physical properties as that of conventional diesel fuel, and can be used either on its own or mixed with conventional diesel in any diesel engine, without having to modify either the ignition system or the fuel injector [10].

Canola oil, soybean oil, palm oil, sunflower oil, cotton-seed oil, waste vegetable oil are a few widely used edible and non-edible oils for biodiesel production. Few reasons which have not led to the commercial production of biodiesel include collection difficulty and high raw material cost and adverse impact on food supplies, which necessitate the need for a new feedstock for biodiesel production [8]. Microalgae use sunlight more efficiently, than other crop plants, to produce oil [11]. According to Chen et al. their oil production capacity is almost one or two times higher than any other energy crop [12].

There are different conversion processes by which microalgae can be converted into different forms of energy [13]; which mainly include thermo-chemical and bio-chemical processes [14]. They can be used to produce a number of different biofuels including vegetable oils, biodiesel, bio-ethanol, bio-syngas, bio-oil, and bio-hydrogen [3]. However, the current research is mostly focused on biodiesel production from microalgal oil [6].

2. About algae

Algae are unique eukaryotic microorganisms, which convert sunlight, water and CO_2 to biomass resource with the process

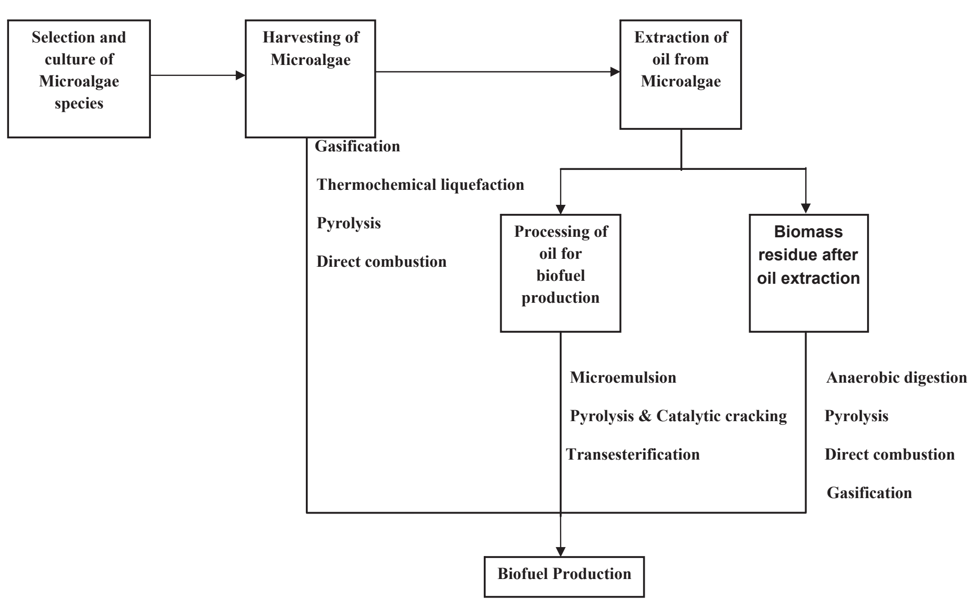


Fig. 1. Different steps involved in producing energy from algae.

called photosynthesis [8,3]. Microalgae are the fastest-growing photosynthesizing organisms [6].

They can be used to generate a wide range of energy products, conversion of algal oil to biodiesel being the most efficient ways [15]. Many algae are exceedingly rich in oil or lipids [8,16], which can be converted to biodiesel [5,17]. Under suitable culture conditions, some microalgal species are able to accumulate up to 50%–70% of oil/lipid per dry weight.

Advantages of microalgae over terrestrial biodiesel feedstock include, short multiplication cycle due to which it can be harvested round-year, and can be cultured in wastewater, thus reducing the fertilizer requirement. They use carbon dioxide as carbon source for growth and produce non-toxic and highly degradable biodiesel, thus help in mitigating environmental concerns. Utilization of microalgae as a source of biodiesel production has both environmental and economic benefits [8].

However, there are a number of technical hurdles which have rendered algal biofuel industry economically unfit [12]. Fast growing strains with high oil yield are the most important requirement for effective biofuel production from algae. Oil extraction methods and conversion technologies also need to be developed and optimized [18].

Looking at the potential of microalgae as a third generation biofuel feedstock, this review collates and presents an overview of current harvesting, oil extraction and biofuels production technologies from microalgae. Fig. 1 shows different steps for biofuel production from microalgae.

3. Microalgal harvesting technologies

After lipid synthesis, for further processing of microalgal biomass to biofuels requires water removal from the algal culture. Harvesting alone, accounts for 20%–30% of the total production cost [19]. Therefore, for mass biodiesel production, efficient harvesting method is very essential [20,2].

Selection of the appropriate harvesting method is of great importance to the economics of biofuels production. The appropriate harvesting method strongly depends upon the characteristics of the microalgae chosen [19], viz. the density and size, as well as the specifications of the desired product [20]. An optimal harvesting method should be species independent, should use less chemicals and energy, and if possible, also release intracellular materials [12].

Dewatering small sized and initial dilute cultures of microalgae is one of the major challenges obstructing the emergence of algae based fuels [2]. Moreover, the cells normally carry negative charge and excess algogenic organic materials are responsible for their stability in a dispersed state [20]. All these factors make economical biomass harvesting difficult, which requires high costs [19,20,12] and energy [2].

Many harvesting strategies like, centrifugation, sedimentation, flocculation, flotation and micro-filtration, can be used to harvest microalgae [19], electrophoresis [20,12] and any combination of these [19].

Microalgae harvesting can generally be divided into a two-step process. In that, the first step is bulk harvesting during which microalgal biomass is separated from the bulk culture. This step concentrates biomass to 2%–7% dry weight. The second step, called thickening, further concentrates the algal slurry. Thickening is more energy intensive than bulk harvesting [12]. Table 1 shows some common harvesting methods and their effectiveness in separating biomass and water.

3.1. Harvesting strategies

3.1.1. Centrifugation

It is the harvesting method which involves centripetal acceleration to separate algal culture into regions of greater and less densities, thereafter the algae and water are separated by draining the excess medium. Centrifugation can also be followed by sedimentation to separate the supernatant [26]. According to Sim, centrifugation is effective in harvesting algae with recovery in excess of 90% and the recovery is directly dependent on the flow throughput [27].

Other laboratory centrifugation tests conducted on pond effluents have also shown similar results. According to them about 80%–90% microalgae (few have also shown it to be 95%–100% efficient [26]) can be recovered within 2–5 min. Rapid and efficient nature of this method makes it one of the most preferred methods for harvesting of algal biomass. However, high energy intensive nature of this method makes it economically unfeasible [19]. The use of centrifugation for harvesting the relatively low concentration (0.04%–0.07%) of total suspended solids in the pond water is restricted by the high cost of power required in handling large quantities of water [27]. Moreover, processing large quantities of culture consumes a lot of time and exposure of microalgal cells to high gravitational, and shear forces can also damage them [12].

3.1.2. Gravity sedimentation

In this method particles in a suspension settle out of a fluid under gravity, and form concentrated slurry and clear liquid above. It is highly energy efficient method [19], and is commonly applied for separation of microalgae from water. Microalgae like *Spirulina*, which settle well by virtue of their high density and large size, can successfully be separated by the sedimentation method. The rate of sedimentation also depends on the induced sedimentation

Table 1
Some common harvesting methods and their effectiveness in separating biomass and water.

Algae	Method	Effectiveness	Conditions	References
Chlorella minutissima	Flocculation followed by sedimentation	60% Recovery efficiency	1g/L of $Al_2(SO_4)_3$ and $ZnCl_2$ and took 1.5 h and 6 h, respectively	[21]
	Centrifugation	16% Dry weight		[13]
	Air sparging assisted coagulation flocculation (ASACF)	7.6% Dry weight		[22]
	Flocculation	Concentrates upto 357 times that of the original	Aluminum sulfate and pH adjustment using hydrochloric acid	[23]
	Centrifugation	15% Dry weight		[24]
Chlorella vulgaris	Gravity sedimentation	60% of biomass was recovered	Settled that biomass, the density of which varied between 0.620 and 0.820 OD at 685 nm and took 1 h.	[25]
	Flocculation	95% of biomass was recovered	Sodium hydroxide was used as flocculant at pH between 11 and 12	[25]
		85%–95% of biomass was recovered	Flocculant used was aluminum sulfate in quantity above 25 mmol/L	[25]

velocity. Microalgal harvesting can be enhanced by sedimentation through lamella separators and sedimentation tanks [12]. However, it is a very slow process [2]. Sedimentation rate can be enhanced by addition of flocculants to the system [12]. But then, flocculants additions have their own pros and cons, which have been discussed later in Section 3.1.4.

3.1.3. Filtration

In this method algae culture runs through filters, which hold back algae and allow the water to pass through them. The process takes place continually until filters contain a thick paste of algae. Microfiltration, dead end filtration, vacuum filtration, pressure filtration, ultra filtration, and tangential flow filtration (TFF) are a few different filtration forms [26].

Larger algae can be effectively recovered by vacuum filtration in combination with filter aid, while micro-filtration or ultra filtrations are effective in recovering smaller algae. However, vacuum and micro-filtration are costly and biomass pumping requirement makes them energy intensive. They also require frequent membrane replacements, due to fouling [19].

Another filtration method called tangential flow filtration is a high rate method. About 70%–89% algae was recovered using this method. Another advantage of TFF is that it maintains the structure, properties and motility of the filtered microalgae [12]. Considering the output and initial feedstock concentration, according to recent studies TFF and pressure filtration are energy efficient harvesting methods [26]. However, membrane replacement and pumping limit large scale harvesting by TFF [12].

Issues like back mixing make simple filtration methods, for example dead end filtration, inadequate for dewatering microalgae culture. However, when used along with centrifugation, give better separation. Filtration methods, in spite being an attractive dewatering option have extensive running costs and hidden pre-concentration requirements [26].

3.1.4. Flocculation

Flocculation is a process in which solute particles in a solution join together to form aggregates called floc [2], which helps in settling [12,28]. Conventional flocculation works by charge dispersion mechanism [19]. Microalgae carry a negative charge [26,12], as a result of adsorption of ions originating from organic matter and dissociation or ionization of surface functional groups [12]. This common negative charge does not let them self-aggregate within suspension [29]. Microalgae can be successfully harvested only by disrupting this stable system [12]. Chemicals called flocculants help to counter this negative charge on the surface of algae [29]. Flocculants displace the negative charge and allow aggregation microalgae cells. Flocculation when combined with sedimentation or filtration increases harvesting efficiency [19]. Flocculation improves the rate of sedimentation of the microalgae by aggregating the dispersed microalgal cells into larger particles and thus, increasing the recovery of biomass [30].

3.1.4.1. Autoflocculation. Autoflocculation is the spontaneous aggregation of particles, resulting in sedimentation of the microalgae [19]. At elevated pH, CO_2 consumed during photosynthesis, precipitates in the form of carbonate salts with algal cells [31]. Carbon limitation or certain abiotic factors can induce autoflocculation [19]. Hence, cultivation of algae in sunlight, with limited CO_2, auto-flocculates algal cells and thus helps in harvesting. NaOH can be added to stimulate autoflocculation, as it can help to obtain the desired pH level [12].

Enhancing natural aggregation/bioflocculation of microalgae for simple gravity settling could prove to be a promising method in terms of effluent quality (total suspened solids) as well as economics of algal biomass recovery for biofuel production [28]. Large colonies (50–200 μm) are often formed by algal species like *Actinastrum, Micractinium, Scenedesmus, Coelastrum, Pediastrum* and *Dictyosphaerium*, which dominate high rate algal ponds used for water treatment [32]. However, more research is needed in this area and the exact mechanisms behind bio-flocculation have yet to be investigated [28].

3.1.4.2. Chemical flocculants. It can be applied over a wide range of microalgal species [12]. In spite of less operating cost, the chemicals added in the process can be hazardous to the environment [26]. According to the nature of the chemicals flocculants can be divided into inorganic, organic/polyelectrolyte flocculants [33]. Table 2 shows the comparison between inorganic and organic flocculants.

3.1.4.2.1. Inorganic flocculants. The negative surface charge on microalgal cells can be neutralized or reduced by addition of iron or aluminum based coagulants [12]. These multivalent salts vary in effectiveness due to their ionic charge [29]. Flocculants, with high charge density, are more effective [2]. Alums are very effective in flocculation of algal biomass during wastewater treatment but they may also later on hinder the oil extraction process of certain algal strains [19].

3.1.4.2.2. Organic flocculants. Organic flocculants or polyelectrolytes are cationic polymers, which physically link cells together. The aggregation strength of the polymer depends on certain specific properties. The organic flocculants to be used will depend on the charge on the algal cells, pH and biomass concentration of the algal culture [26]. High biomass concentrations help frequent cell–cell encounter, thus, help flocculation. Mixing at low level can also perform the same function as that by high biomass concentration, by bringing cells together. But at the same time if shear forces are high, it can also disrupt the flocs. In addition to all the factors mentioned before, functional groups on microalgal cell

Table 2
Comparison of inorganic and organic flocculants.

Parameters	Inorganic flocculants	Organic flocculants
Nature of flocculants	Multivalent salts	Polyelectrolytes/polymers
Key characteristics of an effective flocculant	Increasing molecular weight and charge on the polymers has been shown to increase their binding capabilities [2,12,26]	Flocculants that have a high charge density are therefore more effective
Sensitivity to pH	Coagulation using inorganic coagulants is highly sensitive to pH level [12]	Coagulation using organic coagulants is less sensitive to pH [12,26]
Dosage of flocculants required	A large concentration of inorganic flocculant is needed in order to maintain flocculation efficiency [26,12], thereby producing a large quantity of sludge [28] and may contaminate the end product (for example addition of aluminum and iron salts)	Lower dosages of organic flocculants are required for the flocculation process [26,12], thus producing less quantity of sludge and lesser contamination probabilities
Applicability	Although some coagulants may work for some microalgal species, they do not work for others	Wide range of applications [12,26] i.e. they can be used for larger number of microalgal species

walls are important, because they stimulate the formation of negative charge centers on the cell surfaces [12].

Cationic polyelectrolyte gave better flocculation, whereas no flocculation was found with the anionic polyelectrolyte. Chitosan, commonly used for water purification, can also be used as flocculent [26]. They are biodegradable and do not contaminate the microalgal biomass [12]. However, it is too expensive to be used for economic algae. Further, brackish or saline water requires an additional chemical flocculant to induce flocculation [26]. Polymeric flocculants are generally ineffective in flocculating marine microalgae (with salinity up to 36 g/L), but reducing the salinity improves the flocculation for all cationic polymers [2].

3.1.4.2.3. Combined flocculation. It is a multistep process, which involves more than one kind of flocculant. During a study on marine microalgae, it was found that combination of polyelectrolytes with inorganic flocculants or ozone oxidation followed by addition of flocculants are effective methods of flocculation [12].

Flocculation, when followed by sedimentation or filtration is a cost-effective method of harvesting, as it consumes less power [19].

3.1.5. Electrolytic process

Electrolytic process or electro-coagulation takes place in three steps [12]:

a. Sacrificial electrode undergoes electrolytic oxidation to generate coagulants.
b. Then particulate suspension is destabilized and breaking of emulsion takes place.
c. The destabilized phase again aggregates to form flocs.

In this process microalgae move towards anode, where their surface charge gets neutralized and then the microalgal cells form aggregates. This process is highly efficient and removes about 80%–95% of algal cells [12].

3.1.6. Flotation

Laboratory trials have shown that flotation is suitable for harvesting small, unicellular algae [19]. Flotation is a gravity separation process in which air or gas bubbles attach to solid particles, and then carry them to the liquid surface. Floatation has been found to be more effective and beneficial than sedimentation, in harvesting microalgae. In flotation the algae move upward than downward in case of sedimentation. This favors floatation, as mass cultivation of algae requires high overflow rate. Particles with a diameter even less than 500 μm can be captured by flotation.

On the basis of bubble size, flotation can be divided into dissolved air flotation (DAF), dispersed flotation and electrolytic flotation [12].

3.1.6.1. Dissolved air flotation. In this process pressure of a water stream, pre-saturated with air at excess pressures, is reduced to bubbles of 10–100 μm in size [12]. For more effectiveness of this method, it is important to increase the particulate size of the algal biomass, for which flocculants are added to bind the cells together to facilitate settling. Air bubbles, passed into the solution, adhere themselves onto the particulate mass, and increase their buoyancy and make the algal particles to float to the surface where a compaction zone is formed [27]. It uses chemical flocculants like alums and autoflocculation is achieved by photosynthetically produced oxygen, with bubbles to separate microalgae biomass. DAF removes microalgae more effectively than settling [12]. However, a common problem associated with dissolved air flotation systems is that oversized bubbles break up the floc [27].

3.1.6.2. Dispersed air flotation. In dispersed air flotation bubbles, an air injection system and a high speed mechanical agitator, form bubbles of 700–1500 μm size. The bubbles act by interacting with the negatively charged surfaces of algal cells [12]. The process can be made more effective by reducing the charge of the air bubbles, by addition of cationic surfactant or any other chemical which can give a net positive charge [19].

Ozonation-dispersed flotation is another method of creating charged bubbles. When used to harvest *Chlorella vulgaris*, its cells showed an increase in the lipid content (from 31% to 55%) in the flotation stage. Ozone also causes lysis of the cells and releases biopolymers. These biopolymers act as coagulating agents, and enhance the separation method as well as the lipid extraction process. Contamination in open ponds may prove challenging for Ozonation-dispersed flotation. Moreover, it is an expensive process [19].

3.1.7. Electrophoresis techniques

It is the harvesting process, which does not require any chemical addition. An electric field makes charged algae to go out of the solution. Hydrogen, generated by electrolysis of water, sticks to the microalgal flocs and takes them to the surface. Environmental compatibility, safety, versatility, selectivity, energy efficiency, and cost effectiveness are a few benefits of using this method. Fouling of the cathodes and systems getting damaged by high temperatures as a result of high power requirements, are the main disadvantage of this method [12].

3.2. Integration of different harvesting techniques

Harvesting of microalgal biomass is one of the bottlenecks for biofuel production from microalgae [34]. It can be inferred from the above different harvesting methods that each of them have their own advantages and disadvantages and it also shows that efficiency of one method can be increased if integrated with another method, for example, integrating sedimentation with flocculation [12]. Another such efficient method, which integrated electro-flocculation with dispersed-air flotation, was used for harvesting *Botryococcus braunii* [34]. According to another author, flocculation in combination with flotation or sedimentation followed my centrifugation or filtration is the most energy and cost efficient choice [30].Thus, integration of different methods is an efficient technology for harvesting microalgae.

While undertaking research on harvesting, oil extraction, and refining processes for biofuel production from microalgae, nature and type of microalgal strain should be considered. Shape of algal cells, cell wall structure and oil composition vary from one algal strain to another, even two different cultures of the same strain are not similar in nature [34].

4. Oil extraction and biodiesel production

Harvesting is followed by oil extraction. The extracted lipid is then converted into biodiesel [19]. Direct transesterification of dried biomass has also been reported in some microalgal and fungal species [9].

4.1. Oil extraction

Lipid extraction is done by the physical methods and chemical methods in the form of solvent extractions, or a combination of the two. Method used for extraction should be fast, easily scalable, effective and should not damage the extracted lipids [19].

Not every lipid fraction is suitable for biodiesel production and moreover sometimes non-lipid contents also get extracted along

with lipid contents. Therefore, the extraction process chosen should not only be lipid specific but should also be selective towards desirable lipid fractions (neutral lipids containing mono, di, and trienoic fatty acid chains) [20]. Removing water, beyond 10–30 wt% dry biomasses, is energy intensive [20]. Therefore, if a lipid extraction methodology can be applied to a wet feedstock, it can save a lot of energy [35].

4.1.1. Pre-treatment: cell disruption methods

Depending on the type of biomass, sometimes before oil extraction, pre-treatment of biomass may be required.

Pre-treatment of samples may be required for oil extraction of certain types of biomass [19]. In wet state, after harvesting, lipids can directly be extracted from microorganisms. It is so because the cells need not be homogenized since they are readily broken by suspending in the extracting solvent [36]. Cell disruption is one such pre-treatments method. Cell disruption method will depend on the type of biomass, state of biomass and scale at which it needs to be applied at [19].

Various cell disruption methods are microwave application, sonication, bead beating, autoclaving [19,20], grinding, osmotic shock, homogenization, freeze drying [19] and 10% (w/v) NaCl addition [20].

Microwaves generate high frequency waves, which shatter cells via shock induction. It was recently suggested to be an efficient method for disruption of oil containing plant cells. Sonication, widely used for microbial cells, disrupts both cell wall and membrane by cavitation effect. While in bead-beating, high-speed spinning with fine beads cause mechanical disruption of the cell. Bead-beating has gained success, on both bench and industrial scales [20].

Various methods, including bead-beating, sonication, autoclaving, microwave application, and 10% (w/v) NaCl addition, were experimented for disruption of *Botryococcus sp*., *C. vulgaris* and *Scenedesmus sp.*

Bead-beating and microwave were found be most efficient; specifically in case of *Botryococcus sp*., whereas sonication had the lowest efficiency. On further experimentation on *B. braunii*, not only sonication but bead-beating was also found to be better than other methods like french pressing or lyophilization, and homogenization. Despite its high efficiency, the only drawback of bead-beating is that it is not easily scalable.

When experimented on *C. vulgaris*, microwave oven and autoclaving were found to be most efficient methods, while bead-beating turned out to be the worst method. Microwave can also easily be scaled-up.

For *Scenedesmus sp*., the microwave oven method gave the best results, whereas the efficiencies of sonication, bead-beating, and osmotic shock methods were almost similar. In case of *C. vulgaris* and *Scenedesmus sp*, osmotic shock in spite of being simple and showing results similar to bead-beating, requires longer treatment time (48 h).

Therefore, cell disruption efficiency for lipid extraction in microalgae differs from species to species, and also depends on the employed extraction method [20]. Table 3 shows the performance of various pretreatment methods in extracting lipids.

4.1.2. Lipid/oil extraction methods

Table 4 shows the effectiveness of various oil extraction methods.

4.1.2.1. Solvent extraction method. In this method extraction of algal oil is done with the use of solvents. Lipids have different kinds of interactions, which also need to be broken for effective extraction. Non-polar organic solvents disrupt hydrophobic interactions between non-polar/neutral lipids; polar organic solvents like alcohols disrupt hydrogen bonding between polar lipids. Strong ionic forces, if present, can be disrupted by shifting pH towards more alkaline. Therefore, the choice of solvent depends on the species of microalgae chosen. Further, the solvent should be inexpensive, non-toxic, volatile, non-polar and poor extractor of other non-lipid components of the cell [19].

Soxhlet extraction and Bligh and Dyer's method are the two typically used methods for extraction of lipids from algal biomass. The Soxhlet method uses hexane and the Bligh and Dyer's method uses mixture of chloroform and methanol as solvents to extract lipids [42]. The other solvents include benzene and ether, but hexane has gained more popularity as a chemical for solvent extraction and it is also relatively inexpensive. Recently Ionic liquids have also been explored successfully for extraction of lipids.

4.1.2.1.1. Soxhelt extraction method. Hexane solvent extraction can either be used alone, or it can also be used in combination with the oil press/expeller method. After extracting oil with expeller, the oil from the remaining pulp can be extracted by mixing it with cyclo-hexane. Cyclo-hexane dissolves oil into it and the pulp is filtered out. Then with the help of distillation oil and cyclo-hexane are separated. The two methods (cold press and

Table 3
Performance of various pretreatment methods in extracting lipids.

Algae species	Autoclaving	Bead-beating	Microwave	Sonication	10% NaCl	Reference
Botryococcus sp., *Chlorella vulgaris*, and *Scenedesmus sp.*	5.4%–11.9%	7.9%–8.1%	10.0%–28.6%	6.1%–8.8%	6.8%–10.9%	[37]
Scenedesmus dimorphous		20.5%		21%		[38]
Chlorella protothecoides		18.8%		10.7%		[39]

Table 4
Effectiveness of various oil extraction methods.

Algae species	Method		% of oil recovered	References
Nannochloropsis sp.	SC-CO_2		25	[40]
Spirulina platensis	SC-CO_2		77.9	[41]
Chlorococcum sp.	SC-CO_2		81.7	[35]
Chlorococcum sp.	Soxhlet		45	[35]
Chlorella vulgaris	Ionic liquids	[Bmim] [CF_3SO_3]	12.5	[42]
		[Emim] [$MeSO_4$]	11.9	
Chlorella vulgaris	Bligh and Dyer's method		10.6	[42]

hexane solvent) when used in combination can extract more than 95% of the total oil contained in the algae. Disadvantage of using solvent extraction is the dangers involved in use of the chemicals. Benzene is a carcinogen, while chemical solvents can also lead to explosion hazard [43]. Hexane though has been found to be less efficient than chloroform, is less toxic, has low affinity towards non-lipid contaminants, and has higher selectivity for neutral lipid fractions [35].

4.1.2.1.2. Bligh and Dyer's method. Lam and Lee found Bligh and Dyer method to have highest lipid extraction efficiency [44]. The Bligh and Dyer method of lipid extraction, yields ≥95% of total lipid and further to it, this method can be used for any tissue containing water up to 80%. Therefore, for lipid extraction, the Bligh and Dyer method has been considered for both dry and wet route [45].

The critical ratios of methanol, chloroform and water should be 2:1:1.8 and that of solvent to tissue should be 3:1. After the solvent and culture are mixed, in the given ratio, they are homogenized to form a monophasic system and then re-homogenized with another similar quantity of chloroform. Therefore, the overall ratio of methanol, chloroform and water should be 2:2:1.8 and that of solvent to tissue is [(3+1):1] [45]. Considering the critical ratios, for dry route, since water content is insignificant in comparison to biomass, solvent to tissue ratio of [(3+1):1] should be considered, while for wet route because of high water content, methanol, chloroform and water ratio of 2:2:1.8 should be considered. The homogenization by centrifuge, separates the biphasis layer (lipid dissolved in chloroform and methanol dissolved in water) formed in the process. Thereafter, the lipid is separated from chloroform and methanol from water by fractional distillation [45].

4.1.2.1.3. Ionic Liquids. Ionic liquids (ILs) are salts that consist of relatively large asymmetric organic cations coupled with smaller inorganic or organic anions. The cations generally consist of nitrogen containing ring structure (e.g., imidazolium or pyrinidine) with a broad range of functional side groups, which decide the polarity of the ILs. The anions vary from single ions like chloride, to larger complex ions like $[N(SO_2CF_3)_2]^-$ [46].

They are also known as green solvents and their characters like non-volatile nature and thermal stability; make them an attractive alternative to volatile organic solvents [42]. They possess relatively no vapor pressure, low toxicity and capacity to be tailored for a specific solubility, polarity, electrical conductivity, and relative hydrophobicity [46].

Kim et al. used mixture of ionic liquid [Bmim] [CF_3SO_3] and methanol in volume ratio of 1:1, ionic liquid [Emim] [$MeSO_4$] and methanol in volume ratio of 1:1. Methanol was used to decrease the high viscosity of ionic liquids. The two mixtures of ionic liquids and methanol dissolved algal biomass leaving lipids insoluble. Undissolved lipids, being lighter than the ionic liquids and methanol mixture, floated during the dissolution process after which the lipid phase was separated by centrifugation. On comparison with Bligh and Dyer's extraction, it was found that [Bmim] [CF_3SO_3] and [Emim] [$MeSO_4$] extracted 12.5% and 11.9% of the lipids, respectively, while only 10.6% of lipid was extracted by the Bligh and Dyer's method [42].

The extraction efficiency of lipids is highly dependent on the anion structure of ILs. Generally, hydrophobic and water immiscible ILs such as [Bmim][PF_6] and [Bmim] [Tf_2N] showed a low extraction efficiency, while hydrophilic and water miscible ILs such as [Bmim] [CF_3SO_3], [Bmim] [$MeSO_4$], and [Emim] [$MeSO_4$] showed a high extraction efficiency, with the exception of [Bmim] [Cl] and [Emim] [Ac]. These results can be partially attributed to the solubility of lipids in the ILs. Higher solubility of hydrophobic ILs for lipids can induce the partitioning of lipids to the methanol and IL mixture phase [42].

4.1.2.2. Supercritical carbon dioxide (SC-CO_2) extraction. It is one of the promising green technology methods, which has the potential to displace the traditional organic solvent lipid extraction methods. A typical extraction unit consists of a feed pump for compression and transportation of liquid CO_2 to the extraction vessel, which is installed inside an oven module, and a heated micro-metering valve to depressurize incoming SC-CO_2. Once the oven is heated, the compressed CO_2 enters the heated oven, in a supercritical state and extracts lipid from the microalgae.

Once completely decompressed, CO_2 evaporates as gas to the ambient, and forces the extracted lipid to precipitate out and collect in the adjoining glass vial [35]. Supercritical carbon dioxide has high solvating power and low toxicity. Intermediate diffusion/viscosity properties of the fluid lead to favorable mass transfer equilibrium and this process produces solvent-free extract. High infrastructure and operational cost associated with this process are its main disadvantages [35].

4.2. Procedures for biodiesel production

Presently, the common method of microalgae based biodiesel preparation entails the following steps: lipid extraction from microalgae, followed by removal of excess solvent, and conversion of lipid to biodiesel [8].

Vegetable oil can also directly be used as biodiesel, by blending them in a suitable ratio with conventional diesel. Due to their high viscosity, direct use of vegetable oil in diesel engines in isolation is technically not possible [10]. High viscosity results in poor fuel atomization [47]; low stability against oxidation due to polyunsaturated nature causes low stability against oxidation and subsequent polymerization reactions, low volatility causes incomplete combustion and thus forms high amount of ashes. Therefore, for direct application in diesel engines, vegetable oils must be processed to acquire the necessary properties [10]. Micro-emulsification, pyrolysis (or cracking), and transesterification are few possible processes, which have been discussed below.

4.2.1. Micro-emulsion of oils

Short chain alcohols like ethanol and methanol are used for micro-emulsions. Studies have been conducted on reducing high viscosities of vegetable oils by forming micro-emulsions with short chain immiscible alcohols like methanol and ethanol and ionic or non-ionic amphiphiles [6]. Though micro-emulsion of vegetable oil lowers their viscosity but has been found to result in irregular sticking of injector needle and heavy carbon deposits due to incomplete combustion of the oil [48].

4.2.2. Pyrolysis and catalytic cracking

Biomass pyrolysis is a promising technique for simultaneous production of liquid, activated carbon and gaseous fuels and important chemicals [49]. It is a thermo-chemical process in which the biomass is either heated in the absence of oxygen or is partly combusted in the presence of low oxygen supply [50]. The liquid fuel obtained from pyrolysis has similar chemical components as that of conventional petroleum diesel [51]. The pyrolyzed vegetable oil has low viscosity, high cetane number. They have acceptable amounts of sulfur, water and sediment contents and copper corrosion values, but their carbon residues, ash contents and pour points are not in acceptable range [48].

4.2.3. Transesterification

Micro-emulsification, pyrolysis or catalytic cracking, both are cost intensive and produce a low quality biodiesel. Transesterification is the most usual method to convert oil into biodiesel [10], and is the best choice as fatty acid (m)ethyl esters (biodiesel),

produced by this process have their physical characteristics very close to those of diesel fuel. Moreover, it is a relatively simple process [6].

Transesterfication converts raw and viscous microalgal lipid (triacyleglycerols/free fatty acids) to lower molecular weight fatty acid alkyl esters [19]. The alkoxy group of an ester compound is exchanged by an alcohol (alcoholysis), carboxylic acids (acidolysis) [6] or an ester (interesterification). Only alcoholysis and inter-esterification have gained importance and are used to produce biodiesel [10]. Thus, it is a reaction between the parent oil (triglyceride) and a short chain alcohol, in the presence of a catalyst. Fatty acid methyl esters (FAME) and glycerol are the products of the reaction [19].

Ethanol can be produced by the fermentation process, thus is more renewable, and also less toxic. In spite of this, methanol being cheaper, more reactive and produce more volatile fatty acid methyl esters, is preferred over ethanol [10]. The reaction rate and yield can be improved by use of a suitable catalyst [6]. The catalyst can be acidic, basic or enzymatic in nature [19]. Fig. 2 shows the

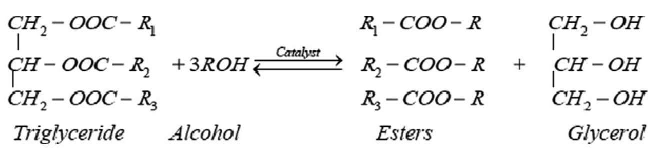


Fig. 2. Transesterification of triacylglycerols with alcohol in the presence of acid, base or enzyme catalyst to give esters (FAME) and glycerol [19].

transesterification reaction of triacylglycerols with alcohol to produce esters (FAME) and glycerol in the presence all the three types of catalyst.

Few examples of the catalyst used for transesterification are: (1) alkaline catalyst (potassium hydroxide, sodium hydroxide and sodium methoxide); (2) acid catalyst (hydrochloric acid, sulfuric acid, and sulfonic acid [10,6], phosphoric acid [10]; (3) enzymatic-catalyst include lipases [10,6]; (4) inorganic heterogeneous catalyst (solid phase catalyst) [10]. Table 5 shows comparison of different catalysis techniques.

4.2.3.1. Base catalysis. High heat of reaction of metallic potassium makes its handling dangerous. Therefore, use metal alkoxides (e.g. sodium methoxide) in methanol are better options than metal hydroxides (NaOH, KOH). Alkaline metal alkoxides, even in small concentrations of 0.5 mol%, are highly active catalysts. In short reaction time of about 30 min they give high yields of about 98%. However, they perform better in absence of water, which makes them inappropriate for industrial processes [52].

4.2.3.2. Acid catalysis. Acid catalyst can be used in combination with base catalyst (two stage process). High fatty acid containing low-cost feedstock like waste oil can be processed by this two stage method. In the first stage, free fatty acids are converted to methyl esters by acid catalyst, and then base catalyst converts the left over triglycerides to methyl esters.

Table 5
Comparison of different catalysis techniques.

Parameters	Base catalysis	Acid catalysis	Enzymatic catalysis
Scale of application	Most widely used process. Currently, practically 100% of biodiesel is produced by the alkaline process	Acid catalysts are rarely used on the industrial scale because of their corrosive nature	Enzymatic technology seems to be starting its application in industrial scale. For large scale production this may not be economically viable due to high enzyme production costs [19,6]
Rate of reaction	It is a faster reaction [19]	The reaction is slow. Speeding up the acid catalyzed reaction requires an increase in temperature and pressure making it prohibitively expensive at large scale [19]	Enzymatic catalyzed reactions are slower than the alkaline catalyst and the risk of enzyme inactivation due to methanol Other benefits include moderate reaction conditions thus less energy intensive [19] and does not even run the reaction to completeness
Effect of alcohol	Since it is reversible reaction, the rate of forward reaction increases with addition of more alcohol	Since it is reversible reaction, the rate of forward reaction increases with addition of more alcohol	It is well known that if methanol is in a relatively high amount with respect to oil, it may inhibit and deactivate a large proportion of lipase. Thus, lower alcohol to oil ratio requires for production [19]
Effect of FFA content	Alkaline transesterification reaction is limited by free fatty acid content (FFA) [19], therefore for alkali transesterification its amount should not exceed a certain limit. Alkaline catalysis is preferred over acid catalysis for oil samples containing FFA below 2.0% [48]	It is suitable for transesterification of oils containing high levels of free fatty acids [19] In any case, acid catalyst is the recommended process when the starting materials are low grade or have a high concentration of free fatty acids. Since it is more corrosive, its yield is lower in comparison to base catalyst.	It is a viable method for parent oils containing high levels of free fatty acids as they can also be converted to alkyl esters [19]
Downstream recovery	FFA are responsible for saponification, leading to consumption of the base catalyst as well as making downstream recovery difficult [10,19] The process also requires the absence of water, which makes them inappropriate for typical industrial processes [6]	No soaps are formed, if the reagents are moisture free [10,19]	Easier product recovery. If lipase is immobilized, it can be easily separated from the reaction mixture by filtration, or when the lipase is in a packed bed photobioreactor (PBR), no separation is necessary after transesterification [19].
Effect on environment	In order to treat the alkaline effluents generated, a lot of water is consumed during washing in the purification steps, means that the alkaline process is not so environmental friendly. Moreover, glycerol, which is a byproduct formed during the reaction, is usually contaminated with alkaline catalysts, thus, its purification to provide an added value to the alkaline process is not easy	It environmental effects are similar to base catalyst	The subsequent separation and purification of biodiesel is easier than with alkaline catalysts. Immobilization also increases the stability of the lipases and the potential for repeated use

Acid catalysts should be preferred when converting microalgal oils to biodiesel. In an experiment on *Chaetoceros mulleri*, in similar conditions from 250 mg of lipid,10 mg of FAME was obtained from acid catalysis (0.6 N hydrochloric acid–methanol catalyst), while only 3.3 mg of FAME was obtained from base catalysis (sodium hydroxide) [10].

Chemical catalyzed transesterification process requires high amount of energy and separation of catalysts from the product. Alkaline water produced during washing needs remediation, while presence of free fatty acids and water result in product loss because of saponification. Even glycerol recovery is difficult [19].

4.2.3.3. Enzymatic transesterification. Enzymatic technology has already been implemented on the industrial scale, especially in China with a capacity of 20,000 t/year. The catalyst used is lipase. These enzymatic biocatalysts are of two types:

a. Extracellular lipases: they are extracted from the live microorganisms like *Mucor miehei*, *Candida antarctica*, *Rhizopus oryzae*, and *Pseudomonas cepacia*, and then purified.
b. Intracellular lipases: they remain either inside or in the cell-producing walls.

Both the above enzymes are immobilized before use. Immobilization eliminates downstream operations of separation and enzyme recycling [10]. Fig. 3 shows the flow diagram for FAME production via enzyme mediated alcoholysis [19].

To lessen the deactivating effect of methanol, uses of solvents have been proposed for both methanol and oil. Solvent like t-butanol has been found to be most suitable for methanol based alcoholysis on an industrial scale. Lipases can be reused repeatedly without any loss in their activity in the reaction system with t-butanol. Another alternative could be a stepwise addition of methanol in the reaction mixture [10].

Glycerol produced as a byproduct of alcoholysis, readily adheres to the surface of immobilized lipase and decrease its enzyme activity. And glycerol removal being a complex process may hinder the continuity of larger scale operations [19]. Large accumulation of glycerol in the reaction mixture may also inhibit the lipase by covering it. Some researchers have suggested in situ removal of glycerol by dialysis, or by extracting it with isopropanol. However, use of t-butanol for transesterification reaction, can be a better option as it dissolves the glycerol into it [10].

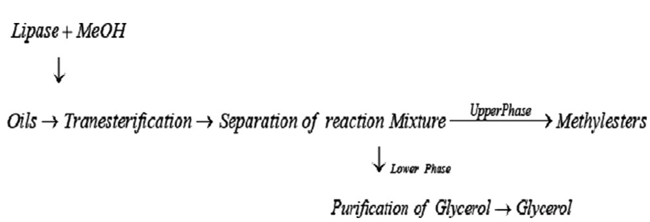


Fig. 3. Flow diagram of enzyme mediated alcoholysis for FAME production [19].

4.2.3.4. Biodiesel production from microalgae species using heterogeneous catalysts. A study on magnesium oxide and calcium oxide as catalyst showed that pure CaO and MgO catalysts were not suitable for transesterification of microalgal lipid. Basic catalysts are the most suitable catalysts for transesterification of oils with low free fatty acids content. Despite CaO and MgO being basic catalysts, they have not been found suitable for the transesterification of microalgal lipid. However, their activity can be increased by mixing them with Al_2O_3, which again is not suitable for transesterification of microalgal lipid when used alone. Among the various mixed ratios, 80 wt% CaO/Al_2O_3 was the most suitable and could be reused at least two times [53].

4.2.4. In situ or direct transesterification

It is a one-step method in which both extraction and transesterification of the algae oil takes place simultaneously in the reactor [9]. It not only reduces the procedure units but also lowers the final biodiesel cost by reducing the overall process cost [8]. It also consumes much less time than the conventional two-step process [54].

After the microalgae are dried, to prevent unwanted soap formation during transesterification, it is crushed into small solid particles [9]. Methanol acts as the extractant as well as reactant. The two simultaneous processes extraction and transesterification, demand solvents with different polarities. Therefore, methanol is mixed with a non-polar solvent in a suitable ratio. Experiments have shown that methanol and methylene dichloride in ratio of 3:1 enhance the efficiency of the extraction. Here methylene dichloride acts as a co-extractor [8].

Results showed that one-step process gave higher methyl ester yield than the conventional two-step method. The biodiesel also had higher HHV. One-step process could also shift the extraction equilibrium and promote the extraction efficiency. It also helped to reduce the overall heat requirement and cost of biodiesel production [8]. Table 6 shows comparison of two-step and direct transesterification.

While conventional conversion route exhibited many inherent disadvantages like operational complexities, high energy consumption and comparatively high cost, which limited its application on commercial scale for biodiesel production from microalgae. Moreover, a lot of waste liquid is formed during purification of the product, disposal of which is another environmental problem [8].

However, the reported in situ transesterification reaction usually used homogeneous acid or alkali as catalyst, which resulted in complexity of products purification and environmental problem

Table 6
Comparison of two-step/conventional and one-step/direct transesterification [8].

Parameters	Two-step transesterification	One-step or direct transesterification
Conversion process	The conventional two-step method involves the extraction of lipid and the removal of excess solvent followed by transesterification of extracted lipid	Both extraction and reaction of the algae oil are performed simultaneously in the reactor, thus simplifying the conversion process by reducing the procedure units
Overall process cost	The overall process cost is high	Reduces the overall process cost
Higher heating value (HHV)	HHV of biodiesel by two-step method was 27.19 MJ/kg	HHV of biodiesel by one-step method was 31.53 MJ/kg
Biodiesel yield	The yield reached only 22.2% through two-step method	The highest methyl ester yield of 28.0% was obtained through one-step method operated at 65 °C for 4 h with 45 mL mixed solvent (methanol/methylene dichloride = 3:1, V/V) and 10% catalyst

unavoidably. To overcome the above problems of transesterification, one-step transesterification was performed to produce biodiesel from (*Nannochloropsis sp.*) microalgae on heterogeneous solid base catalyst (Mg–Zr solid base catalyst), which reduced the process of the product purification and the emission of waste liquid. The catalyst was separated easily from microalgae residue [8].

5. Algae mass after oil extraction/energy recovery from lipid extracted microalgal biomass residues (LMBRs)

LMBRs are rich in carbohydrates (polysaccharides), proteins [55], and pigments [34]. They can further be processed to produce a wide range of biofuels like bio-methane, bio-ethanol, bio-hydrogen and bio-butanol etc. [19]. As discussed in the anaerobic digestion section, the LMBRs can produce a great amount of biogas, which can improve the overall energetic and economic of the energy production system [56]. Lipid extracted algal biomass can also be utilized to produce hydrogen gas. Hydrogen is a clean and efficient energy carrier and forms only water as a byproduct [56].

Apart from biofuel production from microalgae, the bio-refinery approach can be used to produce other valuable products like Docosahexaenoic acid (DHA), carotenoids [19], drugs, food and feed additives [34]. LMBRs could be used as animal feed [56]. Along with the co-production of high value products from micro-algae, both the environmental and economic benefits of biofuel production from microalgae can be enhanced [34].

6. Glycerol byproduct

Glycerol is produced as a byproduct in biodiesel industry. Per 10 L of biodiesel produced, produces 1 L of glycerol. And at the current annual biodiesel production capacity of 9.8 billion liters, 980 million liters of glycerol/yr are produced compared to a demand of only 216 million liters/yr [57]. Once the commercial production of biofuel starts, exploitation of the huge quantity of glycerol could be a major problem. Few other uses of glycerol are, as a carbon source during mixotrophic cultures, and for microbial production of 1,3-propanediol and bio-plastic poly (3 hydroxybutyrate) [34]. Glycerol can also be converted into hydrogen gas [34,57] by anaerobic fermentation.

Per mole of glycerol produces only 3 mol of hydrogen and acetate being the main end product of the fermentation process. Moreover, this being an endothermic reaction additional energy needs to be supplied. Another alternative method called electro-hydrogenesis produces 3.9 mol of hydrogen per mole of glycerol, which is higher than that produced in fermentation process [57].

7. Other techniques of producing energy from algae

There are basically two types of conversion technologies for converting microalgae biomass into biofuels; these are thermo-chemical and biochemical conversion. In thermo-chemical process the organic biomass is thermally decomposed to fuel products. Thermo-chemical conversion techniques include direct combustion, pyrolysis, thermo-chemical liquefaction and gasification [58]. The biochemical conversion techniques include alcoholic fermentation, anaerobic digestion, and photo-biological hydrogen production [59]. Fig. 4 shows different energy production processes from algae.

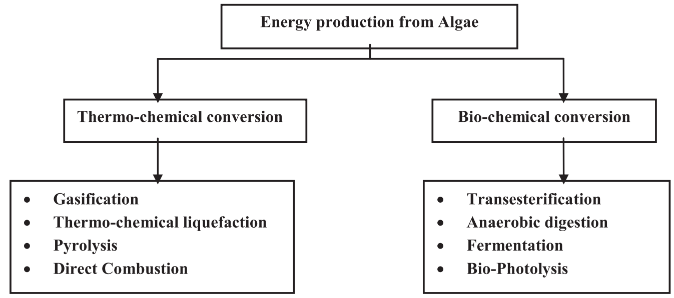


Fig. 4. Different processes for energy production from algae.

7.1. *Thermo-chemical conversion*

In addition of the production of biodiesel, using oils extracted from microalgal cells, various thermo-chemical conversions has been applied for production of energy from algae [19]. Main thermo-chemical processes include liquefaction, pyrolysis and gasification [3].

7.1.1. *Gasification*

It is also known as hydrothermal process [17], during which partial oxidation of biomass at high temperature of around 800–1000 °C, produces mixture of combustible gases. Biomass reacts with oxygen and steam water to produce mixture of gases known as syngas. Syngas consists of gases like methane, hydrogen, carbon dioxide and nitrogen. Syngas can be either directly burned to produce energy or can be used as a fuel to run diesel or gas turbine engines [19].

Gasification is an environmental friendly process of biomass conversion into biofuels. Water is heated above its critical temperature and pressure during which physical properties of water like, dielectric constant, viscosity, density and thermal conductivity of water decrease drastically. The ionic product is almost three times higher than that of normal water. High temperature water behaves as a very good solvent and completely dissolves and breaks the organic compounds. The advantage of this process is that it does not require drying of the high water containing algal biomass and saves a lot of energy, which otherwise would have been used for drying purpose [17].

7.1.2. *Thermo-chemical liquefaction*

In liquefaction process, biomass is converted to liquid fuel [60]. Liquefaction takes place at temperatures between 200–350 °C in the presence of a catalyst [61]. At sub-critical condition of water, biomass breaks into small, reactive and unstable molecules [19] and then re-polymerizes to form wide range of molecular products. Alkali salts, like potassium and sodium carbonate can act to hydrolyze cellulose and hemicelluloses into smaller fragments [62]. In a liquefaction experiment on *B. braunii* at 575 K catalyzed by sodium carbonate, a maximum yield 64% dry weight basis of oil was produced [3]. Depending on the species, liquefaction of microalgae produces between 30%–65% dry weight of oil [19].

Conversion of wet biomass into bio-crude oil is the major advantage of the liquefaction process [19], which saves the energy required in drying the high water containing algae culture [3,16]. Moreover, from energy balance point of view the liquefaction process was found to be net energy producer. When compared to supercritical carbon dioxide method of oil extraction from micro-algae, hydrothermal liquefaction was found to be more effective in producing oil from microalgae [3]. However, the complex nature of the reactors makes them very expensive [19].

From the above it is clear that hydrothermal liquefaction is an effective method of biofuel production from microalgae, but in spite this due to lack of much information about this process, more research in this area is required [3].

7.1.3. Pyrolysis

Pyrolysis is conversion of biomass into bio-oil in the absence of oxygen, in the absence or presence of a catalyst. It is a waste less and pollution free process, during which biomass decomposes into charcoal, condensable organic liquids, acetic acid, acetone, methanol and non-condensable gaseous products [19].

With the increase in the temperature the amount of liquid product increases and that of charcoal decreases [3,16]. Slow pyrolysis produces more charcoal, while fast pyrolysis produces 75 wt% of liquid bio-oil, 15–25 wt% solid charcoal and 10–20 wt% non-condensable gases. Flash pyrolysis, which takes place at around 500 °C with short vapor residence time, produces 95.5% of liquid biofuel. Pyrolysis of microalgae has been found to produce higher quality bio-oil than that obtained from pyrolysis of lignocelluloses [19].

The pyrolysis oil or bio-oil produced by fast pyrolysis is two to three times cheaper than the gasification and fermentation processes. However, due low quality, their use in conventional gasoline and diesel fuel engines is not possible. They have high oxygen content, are highly acidic and also has high water content of about 25–50 wt%. To make them compatible with current fuels they should be deoxygenated. Several upgradation methods include hydro-treating, aqueous-phase processing and zeolite conversion [63].

It has been found that lipid containing biomass produces more bio-oil, and thus, has higher heat balances [19]. Moreover, the extra oil produced from pyrolysis of oil extracted biomass can also reduce the overall production cost.

7.1.4. Direct combustion

The biomass can also be directly combusted in the presence of air, to liberate energy for heating furnace, boilers and steam turbines. The conversion efficiency of biomass to energy is more favorable than that of direct combustion of coal. The major disadvantage of this process is the huge amount of energy required for drying of microalgae culture, which may affect the energy balance. Therefore, in spite of more efficient than coal, the pretreatment cost makes it less viable than coal. The overall efficiency of the process may be improved, if combusted along with coal. Limited data on viability study of combustion of biomass requires further research and development into it [19].

7.2. Biochemical conversion

7.2.1. Anaerobic digestion of microalgae

Anaerobic digestion of biomass takes place in the absence of air. It produces biogas which is a mixture of methane and carbon dioxide [3]. The anaerobic digestion not only converts the residual biomass left after lipid extraction, but also recycles the nitrogen and phosphorous, which are added as a source of fertilizer during the algae culture. It has been found that the methane produced from lipid extracted algal biomass via anaerobic digestion, produces more energy than that obtained from the lipid [64].

Biodegradability of microalgae, due to its biochemical composition and nature of cell wall, formation of toxic ammonia [64,3] due to high protein content (nitrogen content [3]) and presence of sodium in the marine species, which affects the digester performance [64,65,3], are three main bottlenecks which have been identified for this process.

However, the biodegradability can be improved by pretreatment of the biomass by acting on its physicochemical properties [64]. The pretreatment processes may include substrate concentration [65,64], chemical treatments (acids, bases, ozonation), thermal treatment and ultrasonic lysis, which improve the disintegration of the most refractory organic fractions. These pretreatment processes increase methane yield [66].

Among the various pretreatment options, thermal treatment i.e. temperature was found to be most effective. When heated at 100 °C for about 8 h, it was observed that methane production increased by 33%. Further, when cultured in nitrogen limited conditions, it not only increased the lipid content but also reduced the protein content and thus reducing ammonia release during the anaerobic digestion process [64].

This sodium inhibiting effect could be avoided by the use of adapted marine inoculums. Additionally a study underlines the fact that the sodium is less inhibitory in mesophilic conditions than in thermophilic conditions, which limit the energetic consumptions of this step [65].

Theoretical methane yield depends on the composition of the microalgae. Lipid has higher methane production potential in comparison to carbohydrate and protein. Methane yield increases with the increase in the lipid content of the microalgal biomass. However, lipid hydrolyzes slowly in comparison to protein and carbohydrates [64].

The lipid extracted from biomass can be processed to produce biodiesel while the biomass residue can further be processed via the anaerobic digestion process to produce methane, thus increasing the overall energy yield and production economics. However, the potential methane yield is less from the lipid extracted algal biomass, while ammonia production increases, which may strongly limit and jeopardize the process stability [64].

It has been seen that C/N between 20 and 35 enhances the methane yield. Thus, co-digestion of high nitrogen containing substrate with poor nitrogen containing substrate or in other words substrate with high carbon fractions can significantly enhance the methane yield. Moreover, co-digestion also helps to dilute certain toxic compounds and maintain their concentration under their toxic threshold [64].

Production of methane via anaerobic digestion of the raw algae does not require drying of the biomass, and thus can greatly reduce the overall production cost by removing the harvesting and drying cost, which alone is about 20%–30% of the production cost. Further, when harvesting and drying cost combines with the extraction cost, this alone is about 50% of the total production cost. And thus, use of anaerobic digestion process, could avoid a significant cost and reduce the total energy debt [65].

For algal lipid content lower than 40%, the energetic added value, when recovering lipids, is lower than 21% of the recovered energy [24]. Thus, when lipid content of the cell is less than 40%, anaerobic digestion is a better option with respect to energetic recovery and energy balance of the biomass [64].

7.2.2. Fermentation

Fermentation is the process which produces ethanol from sugar and starch containing crops. It has been used commercially on a large scale in many countries. As of now corn, which contains about 60%–70% starch, is the dominant feedstock of the bio-ethanol industry worldwide [16]. Algae can also be used as a feedstock for bio-ethanol production. The algal starch is converted to sugar with the help of enzymes and then by yeast, this sugar can further be converted to bio-ethanol. Initially, starch is released by using mechanical equipments or an enzyme and then the cell are allowed to degrade, after which *Saccharomycess cerevisiae* yeast is added to it to begin the fermentation process. This produces ethanol, which is taken out of the tank and fed to distillation units [3].

7.2.3. Bio-photolysis

Green algae and cyanobacteria can be used to produce biological hydrogen by bio-photolysis of water [67]. Three different ways to produce hydrogen include, direct photolysis, indirect

photolysis and ATP-driven hydrogen-production. During direct photolysis the resulting hydrogen and oxygen are continuously flushed out. Both photosynthesis and water splitting take place simultaneously and produce hydrogen and oxygen. This can be a major safety risk, and also results in extra cost in separating hydrogen and oxygen. Apart from the separation cost, the other major costs include the cost of photobioreactor and hydrogen storage facility [3].

8. Conclusion

Microalgae, due to several advantages such as high oil content and high growth rate, are a potential source of renewable energy and an ideal biofuel candidate. They can be used to generate energy in several ways. By using thermo-chemical processes, oil and gas can be produced, and by using biochemical processes, ethanol, biodiesel and bio-hydrogen can be produced.

Microalgae cultures have high water content, which must be separated in order to produce biofuels. It can be inferred from the above review that there is no single best method of harvesting microalgae. The choice of preferable harvesting technology depends on algae species, size, density and desired end product. Moreover, harvesting and drying of microalgal biomass highly increases the overall operational cost of biofuel production from microalgae. Therefore, in order to produce biofuels from microalgae economically, more research and development is required to find out an efficient and commercially viable harvesting technology.

There are many processes of getting energy from algae, but each of them along with advantages also carries a few disadvantages. Research for few of them is still in very early stages and moreover currently, biofuel production from algae is still very expensive to be commercially viable. Considering the early stage of research and high cost, it can be said that there is still a long way to go to perfect the process of optimizing the algae biofuel manufacturing process.

Therefore, based on the current research inputs, it appears that apart from identifying the most optimal methods to cultivate algae, one also needs to identify the most optimal method for efficient biofuel manufacturing from them. A lot of work is already being done in each of these two aspects, and it is hoped that there will be many more to come soon.

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